

# Morphological parameters and genetic diversity of progenies from seed production areas and unimproved stands of teak (*Tectona grandis* L.f.) in India

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Received: 2012-04-09; Accepted: 2012-08-22  
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**Abstract:** We compared morphological and genetic attributes of progeny obtained from three seed production areas (SPAs) and three corresponding unimproved stands (UIS) of teak (*Tectona grandis* L.f.) at three broad teak growing regions in the state of Karnataka, India. In general, seed morphological parameters such as seed weight, seed size and seed emptiness were significantly superior in SPAs compared to UISs. Seed germination percentage was also higher in seeds from SPAs. Seedling performance measured at two monthly intervals for six months was observed to be superior in SPAs at two of the three regions compared. Although the genetic diversity of progeny of SPAs was lower than UIS at all regions, the values were moderately high. Germination percent was positively correlated with fruit weight and kernel weight per seed, while diversity indices were negatively correlated with fruit weight, fruit diameter and kernel weight per seed. SPAs proved important as a source of moderately improved planting material with no severe threat to the genetic diversity of future plantations.

**Key words:** ISSR; tree improvement; Shannon index; teak plantations; percent polymorphism

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Corresponding editor: Hu Yanbo

## Introduction

Teak (*Tectona grandis* L. f.; Family Verbenaceae) is an important plantation species of the tropics that is extensively planted across 36 tropical countries of Asia, Africa and Latin America (Chandrasekharan 2005). In India, it ranks second only to *Shorea robusta* in terms of growing stock, contributing 4.59% of the total growing stock of wood in the country (SFR 2009). Being long recognized for its excellent timber qualities, teak was one of the earliest species to be prioritized for genetic improvement, when tree improvement programs in India were first initiated following the recommendations of Kedarnath and Matthews (1962). The core activity of the program was selection of superior trees and, as a long term goal, deploying them in clonal seed orchards (CSOs) to produce abundant and genetically improved seed crops for the plantation industry. Presently, since most CSOs are not fully operational, Seed Production Areas (SPAs) were established by culling and upgrading superior stands of teak. These SPAs served as a temporary source of improved seed until CSO became reproductively operational. However, due to inadequate supply of teak propagating materials from CSOs, much of the supply is obtained from SPAs, which are regarded as an intermediate step in the genetic improvement program.

Seeds from SPAs are always thought to be of superior quality compared to those from plantations because they have been obtained from phenotypically superior stands where inferior trees are culled and management practices introduced to boost the gamete exchange only among the superior types within an SPA. Yet, in spite of the long dependence on seeds from SPAs, there has been little research to validate the assumed morphological and genetic superiority of these seeds. The absence of progeny tests of these populations explains this lack of information. Wellendorf and Kaosa-ard (1988) and Kjær and Suangtho (1997) estimated that seedlings from classified seed stands are expected to have at least 8% higher value production than seedlings from unclassified seed. This increased value production originates

from both improved volume production and better stem form. Yet, for germination and number of plantable seedlings, Indira and Basha (1999) observed no significant difference between different sources, which included teak plantations, SPAs, superior trees, and CSOs. Seeds from SPAs had lower germination and numbers of plantable seedlings compared to plantation seeds but both of these parameters outperformed seeds from orchards. Surprisingly, comparative analysis of the genetic structure of progeny from SPAs, CSOs and plantations has not been reported. Maintenance of sufficient genetic variation over generations is crucial for progeny performance.

In this paper, we compared the morphological and genetic characters of progeny obtained from SPAs with those from unimproved stands of teak. We chose three SPAs located near three unimproved plantations, with five years of age difference. We compared fruit, germination, seedling performance and the genetic structure of the two population types.

## Material and methods

### Sampling sites

We selected three sites representing teak growing regions within the state of Karnataka along the Western Ghats range (Table 1). At each site, we chose a control or unimproved stand (UIS) and a seed production area (SPA). The two populations at each site were not separated by more than 2 km and were of the same age. The age of the plantations was ascertained through forest records. In each population, we measured the girth of 30 mother trees that were separated by a minimum distance of 70 m and collected fruits. Fruits from the 30 mother trees were collected during March 2006. Fruits collected from individual trees within each population were subsequently bulked making sure that each mother tree was represented in the total collection.

**Table 1. Details of sites for collection of leaves and fruits**

Regions	Name of the place	Population	Area (ha)	Age (years)	Lat ( $^{\circ}$ N)	Long ( $^{\circ}$ E)	Alt (m)
Northern	Verrampali	UIS	--	55	15° 27'	74° 79'	599
		SPA	20	55	15° 27'	74° 79'	599
Central	Kunehusur	UIS	--	50	14° 17'	75° 39'	711
		SPA	45	50	14° 17'	75° 39'	711
Southern	Devamachi	UIS	--	77	12° 30'	76° 03'	933
		SPA	50	77	12° 30'	76° 03'	933

UIS: Unimproved population; SPA: Seed Production Areas

### Morphological analysis

Morphological analysis included assessment of fruit characteristics, per cent seed germination and seedling growth attributes. These studies were conducted at the Institute of Wood Science and Technology (IWST), Bengaluru (12°58' N and 77°38' E, 900 m). From each population, 100 fruits were sampled from the bulk collections for studying fruit characteristics. Fruits were cleaned

and pericarps removed before recording fruit diameter and fruit weight. Fruits were then opened to check for emptiness as evidenced by empty locules. Kernels wherever present were extracted, counted, weighed and the number of filled locules was divided by the total number of kernels to yield percentages of filled/empty kernels.

For germination studies, 500 fruits (hereafter also referred to as seed) from each source were subjected to pre-germination treatment with cow dung slurry (Chacko et al. 1997). Pre-treated seeds were sown in Randomized Block Design (RBD) at the nursery of IWST, Bengaluru, with five replications and six seed sources. Number of germinated seeds was monitored daily for 40 days beginning 10 days after sowing and germination percent was calculated after the 40<sup>th</sup> day of monitoring.

Seedlings from germination experiments were transplanted into 600 cm<sup>3</sup> root trainers. The transplanting medium consisted of sand, soil and compost (in 2:3:5 ratio) and was enriched with neem cake, single super phosphate (SSP), phorate and Di ethane M45. The seedlings were initially kept inside a polyhouse to minimize transplanting shock. After a week, they were transferred outdoors. The number of seedlings maintained in different populations ranged between 50 and 80 seedlings depending on the extent of mortality. Seedling height and collar diameter were recorded at two month intervals from August 2006 to January 2007.

For all morphological parameters, comparisons were made between UIS and the corresponding SPA. Means and standard deviations were calculated and Student's t-test was used to quantify differences between populations for fruit and growth parameters. Germination percent was compared between populations using the Chi Square test.

### Genetic analysis

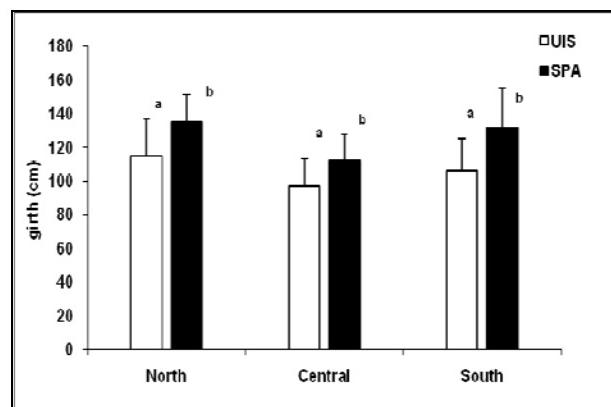
For genetic analysis, leaf samples were collected from seedlings maintained at the nursery for analysis of growth parameters. From each population 30 seedlings were randomly selected and leaf samples collected. A total of 180 samples (6 populations) were subjected to DNA extraction following the CTAB (Cetyl trimethylammonium bromide) extraction method (Doyle and Doyle 1987). DNA was quantified based on the spectrophotometric measurement (Implen Model: Nanophotometer TM, P 300) of UV absorbance at optical density (OD) 260 nm and was diluted to working concentration (20 ng). Genetic analysis was carried out employing DNA based Inter Simple Sequence Repeat (ISSR) molecular markers. PCR amplification (Eppendorf Model: Eppendorf PCR Thermocycler Mastercycler Personnel) was carried out in a 15 µl reaction mixture containing template DNA (20 ng), primer (0.3 µM), *Taq* polymerase (0.5 units), 10× assay buffer and dNTPs (1 mM). The reaction conditions consisted of initial denaturation at 94°C for 4 min., followed by 35 cycles comprising of a denaturation step at 94°C for 5 s, an annealing step at 50°C for 5 s and an extension step at 72°C for 2 min. The cycling program was terminated by a final extension step at 72°C for 20 min. A total of 20 ISSR primers were screened, of which we selected 10 primers (UBC 807, UBC 808,

UBC 809, UBC 811, UBC 818, UBC 826, UBC 827, UBC 866, UBC 868, UBC 898) that gave consistent results and higher number of polymorphic bands. Amplified PCR products were separated on a 1.5% agarose gel stained with ethidium bromide (0.5 µg/ml). The gel was visualized under a UV light and captured using Herolab Gel Documentation Unit (Herolab, Germany; Model: E.A.S.Y Win32).

Binary coding was used to score the gels (Wendell and Weeden 1989). Presence of a PCR-amplified product was scored as 1 and its absence as 0. Based on the absence or presence of amplified products, the per cent polymorphism and Shannon information index was estimated using population genetics software POPGENE version 1.32 (Yeh and Boyle 1997).

## Results

The mean girth of sampled parent trees was significantly higher in SPAs than in UISs in all the three regions (Fig. 1). Trees in SPAs of northern and southern regions had larger mean girth ( $134 \pm 5.34$  cm and  $132.14 \pm 4.25$  cm, respectively) than trees in the SPA from the central region ( $113.05 \pm 6.03$  cm). In all three regions, SPAs produced bigger fruits with higher mass compared to their respective UISs (Table 2). Mean kernel weight per seed was, however, significantly higher ( $0.020 \pm 0.005$  g) only in the southern SPA when compared to UIS fruits ( $0.019 \pm 0.007$  g). The per cent fruit emptiness was lower in SPAs compared to their corresponding UISs in the central and southern regions (Table 2), while it was higher in the northern region (45%). Germination percent was significantly higher in fruits from SPAs in the northern and southern regions ( $21.69 \pm 2.83$  and  $60.31 \pm 7.24$ ) compared to their corresponding UISs ( $14.43 \pm 1.47$  and  $50.65 \pm 2.31$ ). Germination percent was similar for the SPA and UIS in the central region.



**Fig. 1** Mean girth of teak trees in three unimproved stands (UIS) and corresponding seed production areas (SPA) from three different regions of Karnataka (different alphabets indicate t-test significance  $p=0.05$ )

We measured seedling height and collar diameter bi-monthly for six months to compare the early growth performance of progenies of SPAs and of UISs. At the northern and central sites, the progeny performance was significantly superior in SPAs at all periods of observation (Table 3). At the end of the observation period, the mean seedling height and collar diameter of SPA progeny from the northern region were  $8.83 \pm 1.92$  cm and  $4.12 \pm 0.90$  mm, respectively, compared to  $6.29 \pm 1.46$  cm and  $3.43 \pm 0.89$  mm, respectively, for progeny of the northern UIS. Central region SPA progeny measured  $7.81 \pm 1.62$  cm for seedling height and  $4.03 \pm 0.85$  mm for collar diameter compared to  $6.51 \pm 1.73$  cm and  $3.27 \pm 0.82$  mm, respectively, for UIS progeny at the end of six months. No significant differences were found between southern SPA and UIS progeny for any growth parameter at any observation period (Table 3).

**Table 2.** Fruit parameters for unimproved stand (UIS) and seed production area (SPA) populations of teak within each region (North, Central and South) of Karnataka

Population	Diameter (mm)	Weight (g)	Kernel weight /fruit (g)	Emptiness (%)	Germination (%)
Northern					
UIS	$11.47 \pm 1.15$	$0.50 \pm 0.11$	$0.014 \pm 0.004$	30 (33.50)	$14.43 \pm 1.47$ (6.25)
SPA	$12.90 \pm 1.66$	$0.60 \pm 0.15$	$0.016 \pm 0.004$	45 (43.09)	$21.69 \pm 2.83$ (13.75)
T test ( $p=0.05$ )	0.00	0.00	ns	--	11.25*
Central					
UIS	$12.13 \pm 1.42$	$0.57 \pm 0.13$	$0.018 \pm 0.005$	40 (39.90)	$34.61 \pm 3.14$ (31.75)
SPA	$13.40 \pm 1.84$	$0.64 \pm 0.17$	$0.019 \pm 0.009$	32 (34.80)	$32.17 \pm 1.86$ (28.00)
T test ( $p=0.05$ )	0.001	0.00	ns	--	ns
Southern					
UIS	$12.81 \pm 1.73$	$0.63 \pm 0.21$	$0.019 \pm 0.007$	25 (30.17)	$50.65 \pm 2.31$ (56.25)
SPA	$13.84 \pm 1.77$	$0.77 \pm 0.24$	$0.020 \pm 0.005$	20 (26.62)	$60.31 \pm 7.24$ (67.75)
T test ( $p=0.05$ )	0.00	0.00	0.01	--	4.266*

\*Chi Square test = 0.05; ns: non significant at  $p=0.05$ ; Figures inside parenthesis represent arcsine transformed values.

**Table 3.** Growth parameters for unimproved stand (UIS) and seed production area (SPA) populations of teak within each region (North, Central and South) of Karnataka

Population	Seedling height (cm)			Seedling collar diameter (mm)		
	2 <sup>nd</sup> month	4 <sup>th</sup> month	6 <sup>th</sup> month	2 <sup>nd</sup> month	4 <sup>th</sup> month	6 <sup>th</sup> month
North						
UIS	3.65 ± 1.36	5.63 ± 1.57	6.29 ± 1.46	1.93 ± 0.58	3.08 ± 0.72	3.43 ± 0.89
SPA	5.14 ± 1.30	7.72 ± 1.74	8.83 ± 1.92	2.47 ± 0.54	3.77 ± 0.81	4.12 ± 0.90
T test (p=0.05)	0.00	0.00	0.00	0.00	0.00	0.00
Central						
UIS	3.56 ± 1.21	5.77 ± 1.61	6.51 ± 1.73	1.89 ± 0.43	2.95 ± 0.66	3.27 ± 0.82
SPA	5.11 ± 1.34	7.13 ± 1.52	7.81 ± 1.62	2.83 ± 0.58	3.72 ± 0.73	4.03 ± 0.85
T test (p=0.05)	0.00	0.00	0.00	0.00	0.00	0.00
South						
UIS	4.71 ± 1.56	6.63 ± 1.53	7.37 ± 1.37	2.34 ± 0.62	3.52 ± 0.80	3.79 ± 0.85
SPA	4.36 ± 1.10	6.57 ± 1.43	7.38 ± 1.45	2.41 ± 0.63	3.61 ± 0.89	3.90 ± 1.00
T test (p=0.05)	ns	ns	ns	ns	ns	ns

ns: non significant at  $p=0.05$

Genetic diversity of all three regional populations as measured by percent polymorphism and Shannon Index revealed higher values for progeny from UISs as compared to corresponding SPAs (Table 4). A large difference in percent polymorphism was observed between the UIS (64%) and SPA (43.33%) population of the central region.

Fruit weight was positively correlated with fruit diameter ( $r^2 = 0.873$ ; d. f. = 4;  $p < 0.05$ ) and kernel weight per seed ( $r^2 = 0.702$  d. f. = 4;  $p < 0.05$ ). Germination percent was positively correlated with fruit weight ( $r^2 = 0.737$  d. f. = 4;  $p < 0.05$ ) and kernel weight per seed ( $r^2 = 0.797$  d. f. = 4;  $p < 0.05$ ). Shannon diversity index was negatively correlated with fruit weight ( $r^2 = -0.835$  d. f. = 4;  $p < 0.05$ ), fruit diameter ( $r^2 = -0.857$  d. f. = 4;  $p < 0.05$ ) and kernel weight per seed ( $r^2 = -0.756$  d. f. = 4;  $p < 0.05$ ). No significant

relation was found between growth parameters and any of the fruit characters or Shannon diversity index (Table 5).

**Table 4.** Genetic diversity parameters for progeny populations of unimproved stands (UIS) and seed production areas (SPAs) of teak in three different regions

Region	Population	Polymorphism (%)	Shannon index
Northern	UIS	69.33	0.319 ± 0.255
	SPA	62.67	0.292 ± 0.270
Central	UIS	64.00	0.304 ± 0.265
	SPA	43.33	0.240 ± 0.276
Southern	UIS	62.67	0.254 ± 0.250
	SPA	52.00	0.224 ± 0.253

**Table 5.** Correlation analysis between different fruit characters, germination percentage, growth parameters and genetic diversity index

	Diameter	Weight	Kernel weight/ seed	Emptiness (%)	Germination (%)	SH2	SH4	SH6	CD2	CD4	CD6
Diameter	-	-	-	-	-	0.465	0.433	0.382	0.647	0.649	0.589
Weight	0.873*	-	-	-	-	0.17	0.157	0.138	0.314	0.362	0.303
Kernel weight/ seed	0.644	0.702*	-	-	-	0.102	0.054	0.031	0.239	0.146	0.095
Emptiness (%)	0.123	0.302	0.22	-	-	0.007	0.073	0.109	0.013	0.009	0
Germination (%)	0.48	0.737*	0.797*	0.468	-	0.016	0.002	0	0.058	0.071	0.034
Shannon Index	-0.857*	-0.835*	-0.756*	0.403	-0.647	0.309	0.187	0.131	0.548	0.046	0.381

\*significant at  $p=0.05$  and d.f. = 4; SH: Seedling height; CD: Collar diameter; 2, 4, 6: month

## Discussion

The objective of introducing intercultural operations such as thinning, fertilization and irrigation in SPAs is to encourage growth of the retained trees and enhance seed production. In our study plots, for SPAs and UIS of the same age, mean tree girth in SPAs was significantly higher than in UISs (Fig. 1), and seed quality (fruit weight and diameter) were significantly superior in SPAs (Table 2). This might be explained as a combined effect of

enhancement of the temperature regime and increased light, nutrient, and water availability, all of which improved after neighbouring trees were removed (Jackson and Sweet 1972), and resulted in production of significantly better seed. Mutnal et al. (2003) showed that teak tree diameter had significant effects on seed characters such as seed weight and diameter.

In teak, low seedling production due to high seed emptiness has been the greatest impediment to meeting the demand for planting stock. For this parameter, there was no consistency in

ranking between SPAs and UISs, and differences observed between the plots were not very large (Table 2). Although Banik (1977) observed that seedless fruits were less common in higher fruit diameter classes, we found no significant correlation between per cent emptiness and fruit dimension (Table 5). While some have contested the lack of sufficient pollination (Hedegart 1973) and low pollen ovule ratios (Indira and Mohanadas 2002) as reasons for low seed setting, others conclude that intrinsic factors such as nutrient resource scarcity (Karmacharya and Singh 1992; Palupi and Owen 1998), dominance hierarchy due to inhibitory chemicals produced by dominant seeds (Tamas et al. 1979; Huf and Dybing 1980; Ganeshiah and Uma Shanker 1988), and fungal infections (Mohanana et al. 2005) could lead to seed abortion. Recently, Lyngdoh et al. (2010) reported negative association between genetic diversity of teak populations and percent emptiness of fruits.

Germination percent was significantly higher in SPAs compared to UISs in the northern and southern regions. Seedlings from SPAs were also found to have superior growth parameters compared to UISs in the north and central regions throughout the observation periods (Table 3). This result contrasts with the findings of Indira and Basha (1999), who reported that teak seeds from unimproved stands had higher germination percentages and performed better than seeds from SPAs. However, their report was based on comparisons of SPAs and UISs from different regions, which allowed site variation factors to influence their results. We attribute the differences in germination percentages between fruits of SPAs and UISs from the same region to larger fruit size and higher kernel weight per seed of fruits from SPAs (Table 3). Earlier studies on teak revealed that seed germination potential is associated with seed dimensions (Eidmann 1934; Dabral 1976; Banik 1977; Jayashankar et al. 1999; Sivakumar et al. 2002; Sudhakaran and Jijesh 2008). Studies conducted in several species revealed that the seed character of a species can be indicative of the quality of the seed as expressed by germination percent (Toon et al. 1990). Confirming earlier studies, we recorded positive correlation between fruit weight, kernel weight per fruit and germination percentage. However we recorded no significant correlation between any of the fruit characters or germination percentage with growth parameters.

Progeny populations of SPAs had lower genetic diversity than the UIS progenies in all regions (Table 4). We found no earlier study comparing levels of genetic diversity of progenies obtained from seed stands or SPAs with those from unimproved stands of teak. However, comparisons of the genetic diversity of progenies and their parent populations in thinned stands (Adams et al. 1998; Medri et al. 2003) have shown a decline in the former. One possible reason could be elimination of inferior phenotypes in SPAs, which might reduce the variation through mating between similar individuals.

Given the importance of genetic diversity to ensuring evolutionary flexibility (Yanchuk 2001), it is recommended that seed lots used for plantation establishment should have adequate genetic diversity to ensure good survival and growth of trees (Nicodemus et al. 2005). Although we observed reduced genetic diversity among progenies of SPAs, the diversity level *per se*

was not low (Table 4). According to Anthony et al. (2001), a total gene diversity index can be considered high if it is above 0.25. This was perhaps the reason we observed no significant relationship between genetic diversity of populations and growth performance. Yet a negative correlation was found between genetic diversity and fruit characters. Lyngdoh et al. (2010) reported that genetically diverse parent populations of teak produced seeds of inferior quality in terms of percent seed emptiness and germination percentage. They reasoned that high genetic diversity may be associated with high flowering asynchrony within the populations which could in turn affect seed production.

Teak has been under cultivation in India for over 150 years yet improvement is still in its infancy. Plantations are still raised with seeds procured from unknown and unimproved sources, and productivity has been low (Pandey and Brown 2000). Currently, apart from clonal orchards, the alternate source for improved seed is the seed production areas. This is perhaps the first evidence which categorically compares the genetic and morphological quality of propagules obtained from SPAs with those from UISs. We conclude that SPAs can be a potential source of improved planting materials that will cause no drastic reduction in the diversity of planting material. While these stands might continue to contribute significantly to the seed supply, intensive management of these stands would greatly increase the seed output. However, steps would be necessary to ensure maintenance of genetic diversity in SPA populations and their progenies. For example, low-intensity improvement would be useful to capture the high within-population variation present in teak (Changtragoon and Szmidt 2000; Nicodemus et al. 2003). This would also encourage production of out-crossed seeds by maintaining adequate effective population size (Varghese et al. 2008). Harwood et al. (1996) opined that seed production areas of wide genetic base, preferably from a large number of carefully selected unrelated trees would be a good resource for scientific domestication of teak.

### Acknowledgement

We thank Director, Group Coordinator, and Head of Department, Tree Improvement and Propagation Division, Institute of Wood Science and Technology, Bangalore for providing the facilities. We thank Council for Scientific and Industrial Research (CSIR) for providing scholarship to the first author. Assistance and field support provided by research wing of Karnataka Forest Department is acknowledged.

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